

U.S. Ser. No. 09/581,861
Group Art Unit 1639

Amendment to the Claims:

Please amend claims 1 and 57 and cancel without prejudice or disclaimer claims 2,3, 44, 51 and 56. The following listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A recombinant yeast cell which comprises:
 - a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell; and
 - a chimeric G protein subunit which comprises ~~selected from the group consisting of:~~
 - ~~_____ a non-naturally occurring G protein subunit which comprises a sequence from a heterologous G protein subunit in which at least one amino acid substitution has been introduced compared to the wild type sequence;~~
 - ~~_____ an endogenous STE 18 subunit operably linked to a polypeptide of a heterologous G γ subunit;~~
 - ~~_____ an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit; and~~
 - an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different;

U.S. Ser. No. 09/581,861
Group Art Unit 1639

such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

2. (Cancelled)

3. (Cancelled)

4. (Withdrawn) The yeast cell of claim 1, wherein said chimeric G protein subunit comprises a non-naturally occurring G protein subunit which comprises a sequence from a heterologous G protein subunit in which at least one amino acid substitution has been introduced compared to the wild type sequence.

5. (Withdrawn) The yeast cell of claim 1, wherein said chimeric G protein subunit comprises an endogenous STE 18 subunit operably linked to a polypeptide of a heterologous G γ subunit.

6. (Withdrawn) The yeast cell of claim 4, wherein said non-naturally occurring G protein subunit is a mutant mammalian G α subunit selected from the group consisting of: G α 16(S270P); G α s(D229S); G α s(D229V); G α s(N254D); G α s(S286P); G α s(E10K); G α i2-GaoB(S280P); G α 12(Q229L); G α 12(G228A); and G α i2(S288P).

7. (Withdrawn) The yeast cell of claim 4, wherein said non-naturally occurring G protein subunit is a yeast-mammalian G protein subunit chimera comprising a first polypeptide

U.S. Ser. No. 09/581,861
Group Art Unit 1639

from a yeast G protein subunit and a second polypeptide from a mutant mammalian G protein subunit.

8. (Withdrawn) The yeast cell of claim 7, wherein said second polypeptide comprises a mutant mammalian $G\alpha$ subunit selected from the group consisting of: $G\alpha$ 16(S270P); $G\alpha$ 5(D229S); $G\alpha$ 5(D229V); $G\alpha$ 5(N254D); $G\alpha$ 5(S286P); $G\alpha$ 5(E10K); $G\alpha$ 12-G α oB (S280P); $G\alpha$ 12 (Q229L); $G\alpha$ 12 (G228A); and $G\alpha$ 12 (S288P).

9. (Withdrawn) A yeast cell comprising a chimeric G protein subunit, said chimeric G protein subunit comprising a first polypeptide from a yeast G protein subunit and a second polypeptide from a heterologous G protein subunit, wherein said first polypeptide is a polypeptide from yeast STE 18.

10. (Withdrawn) The yeast cell of claim 9, wherein said first polypeptide is from yeast STE 18 and said second polypeptide is from a heterologous G protein γ subunit.

11. (Withdrawn) The yeast cell of claim 10, wherein said heterologous G protein subunit is mammalian.

12. (Withdrawn) The yeast cell of claim 11, wherein said heterologous G protein subunit is human.

13. (Withdrawn) The yeast cell of claim 12, wherein at least one of said first and second polypeptides comprises a naturally occurring amino acid sequence.

U.S. Scr. No. 09/581,861
Group Art Unit 1639

14. (Withdrawn) The yeast cell of claim 12, wherein at least one of said first and second polypeptides comprises a non-naturally occurring amino acid sequence.

15. (Withdrawn) The yeast cell of claim 9, further comprising a heterologous G protein coupled receptor, which receptor is functionally integrated into the yeast cell.

16. (Withdrawn) The yeast cell of claim 15, wherein said chimeric G protein subunit demonstrates enhanced coupling to the heterologous G protein coupled receptor when compared to that demonstrated by an endogenous yeast G protein subunit.

17. (Withdrawn) The yeast cell of claim 10, wherein said second polypeptide is derived from the human G γ 2 subunit.

18. (Withdrawn) The yeast cell of claim 17, wherein said second polypeptide comprises the amino acid sequence Arg Glu Lys Lys Phe Phe (amino acids 19-24 of SEQ ID NO: 33).

19. (Withdrawn) The yeast cell of claim 18, wherein said second polypeptide comprises the sequence shown in SEQ ID NO: 33.

20. (Withdrawn) The yeast cell of claim 9, further comprising a second chimeric G protein subunit, said second chimeric G protein subunit comprising a first polypeptide from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit, wherein said second chimeric G protein subunit is different from said first chimeric G protein subunit.

U.S. Ser. No. 09/581,861
Group Art Unit 1639

21. (Withdrawn) The yeast cell of claim 20, wherein said second polypeptide of said second chimeric G protein subunit is from a protein selected from the group consisting of: a mammalian G α subunit, a mammalian G β subunit, and a mammalian G γ subunit.

22. (Withdrawn) The yeast cell of claim 15, wherein an endogenous yeast pheromone system receptor protein is not produced in functional form.

23. (Withdrawn) The yeast cell of claim 15, further comprising an indicator gene that produces a detectable signal upon functional coupling of the heterologous G protein coupled receptor to the G protein.

24. (Withdrawn) The yeast cell of claim 9, wherein the cell is a *Saccharomyces cerevisiae* cell.

25. (Withdrawn) The yeast cell of claim 15, wherein said heterologous G protein coupled receptor is an orphan receptor.

26. (Withdrawn) The yeast cell of claim 4, further comprising an indicator gene that produces a detectable signal upon functional coupling of the heterologous G protein coupled receptor to the G protein.

27. (Withdrawn) An assay to identify compounds capable of modulating the dissociation of G α and G $\beta\gamma$, comprising the steps of:

- (i) providing a yeast cell according to claim 15,

U.S. Ser. No. 09/581,861
Group Art Unit 1639

(ii) contacting the yeast with a test compound; and

(iii) identifying compounds which induce a change in a detectable signal in the yeast cell, wherein said detectable signal indicates dissociation of $G\alpha$ and $G\beta\gamma$.

28. (Withdrawn) The assay of claim 27, wherein said test compound is from a library of non-peptidic organic molecules.

29. (Withdrawn) A method for identifying a compound which modulates a heterologous G protein coupled receptor, comprising:

(i) providing a first, second, third, and fourth yeast cell, each cell comprising:
(a) a G protein, wherein:

1) the first yeast cell comprises a first chimeric G protein subunit comprising a first polypeptide from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit;

2) the second yeast cell comprises a second chimeric G protein subunit comprising a first polypeptide derived from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit, said second chimeric G protein subunit being different from said first chimeric G protein subunit;

3) the third yeast cell comprises a third chimeric G protein subunit comprising a first polypeptide from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit, said third chimeric G protein subunit being different from said first and second chimeric G protein subunits; and

U.S. Ser. No. 09/581,861
Group Art Unit 1639

4) the fourth yeast cell comprises an endogenous yeast Gpa1 G protein subunit;

(b) an expressible gene construct encoding a heterologous G protein coupled receptor (GPCR) which couples to the yeast pheromone response pathway; and

(c) an indicator gene that produces a change in a detectable signal upon functional coupling of the heterologous GPCR with the G protein;

(ii) contacting the first, second, third, and fourth yeast cells with a test compound; and

(iii) determining whether the test compound induces a change in a detectable signal in at least one of the first, second, third, or fourth yeast cells to thereby identify a compound which modulates a heterologous GPCR.

30. (Withdrawn) The assay of claim 29, wherein at least one of said first, second, or third yeast cells comprises a fourth chimeric G protein subunit, said fourth chimeric G protein subunit being different from the first, second, or third chimeric G protein subunit expressed by the first, second, or third yeast cell, respectively.

31. (Withdrawn) The assay of claim 29, wherein at least one of said first, second, or third chimeric G proteins comprises a first polypeptide from yeast Gpa1 and a second polypeptide from a mammalian G protein α -subunit.

U.S. Ser. No. 09/581,861
Group Art Unit 1639

32. (Withdrawn) The assay of claim 31, wherein said second polypeptide is from a mammalian $G\alpha i$ subunit.
33. (Withdrawn) The assay of claim 31, wherein said second polypeptide is from a mammalian $G\alpha 16$ subunit.
34. (Withdrawn) The assay of claim 30, wherein said second polypeptide is from a mammalian $G\alpha s$ subunit.
35. (Withdrawn) The assay of claim 29, wherein the first chimeric G protein subunit comprises a polypeptide from mammalian $G\alpha 12$, the second chimeric G protein subunit comprises a polypeptide from mammalian $G\alpha 16$, and the third chimeric G protein subunit comprises a polypeptide from mammalian $G\alpha s$.
36. (Withdrawn) The assay of claim 35, wherein the second chimeric G protein subunit comprises $G\alpha 16(S270P)$ and the third chimeric G protein subunit comprises $G\alpha s(D229S)$.
37. (Withdrawn) The assay of claim 29, wherein each of said first, second, and third yeast cells further comprises a fourth chimeric G protein subunit, said fourth chimeric G protein subunit comprising a first polypeptide from yeast STE 18 and a second polypeptide from a mammalian G protein γ subunit.
38. (Withdrawn) The assay of claim 29, wherein the first, second, third, and fourth yeast cells are contacted with each member of a library of test compounds.

U.S. Ser. No. 09/581,861
Group Art Unit 1639

39. (Withdrawn) The assay of claim 37, wherein each member of said library is a non-peptidic organic molecule.

40. (Withdrawn) The assay of claim 29, wherein said first, second, third, and fourth yeast cells are *Saccharomyces cerevisiae* cells.

41. (Withdrawn) The assay of claim 29, wherein the indicator gene that gives rise to a detectable signal is selected from the group consisting of: β galactosidase, alkaline phosphatase, horseradish peroxidase, exoglucanase, luciferase, BAR1, PHO5, green fluorescent protein, and chloramphenicol acetyl transferase.

42. (Withdrawn) The assay of claim 29, wherein the indicator gene that gives rise to a detectable signal is selected from the group consisting of: HIS 3, β galactosidase, and green fluorescent protein.

43. (Withdrawn) The assay of claim 29, wherein said heterologous G protein coupled receptor is an orphan receptor.

44. (Cancelled)

45. (Withdrawn) A recombinant yeast cell which comprises:
a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said

U.S. Ser. No. 09/581,861
Group Art Unit 1639

heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell;

a chimeric G protein subunit comprising an endogenous yeast G α 1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and

a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of the receptor expressed in the cell membrane; and wherein the heterologous polypeptide is expressed at a sufficient level such that modulation of the signal transduction activity of the receptor by the heterologous polypeptide provides a detectable signal.

46. (Withdrawn) A recombinant yeast cell which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell;

a chimeric G protein subunit comprising an endogenous yeast G α 1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell;

U.S. Ser. No. 09/581,861
Group Art Unit 1639

a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of the receptor expressed in the cell membrane; and

a reporter construct that is activated by the pheromone response pathway; wherein the heterologous polypeptide is expressed at a sufficient level such that modulation of the signal transduction activity of the receptor by the heterologous polypeptide provides a detectable signal mediated by the reporter construct.

47. (Withdrawn) A recombinant yeast cell which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell;

a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and

a mutation in at least one gene selected from the group consisting of *FAR1*, *SST2*, *BAR1*, *SVG1*, *STE2*, *STE3*, *STE14*, *MFa1*, *MFa2*, *MFa1* and *MFa2*; and wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

48. (Withdrawn) A recombinant yeast cell which comprises:

U.S. Ser. No. 09/581,861
Group Art Unit 1639

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell;

a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell;

a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of the receptor expressed in the cell membrane;

a reporter construct that is activated by the pheromone response pathway; and

a mutation in at least one gene selected from the group consisting of *FAR1*, *SST2*, *BARI*, *SVG1*, *STE2*, *STE3*, *STE14*, *MFa1*, *MFa2*, *MFa1* and *MFa2*;
wherein the heterologous polypeptide is expressed at a sufficient level such that modulation of the signal transduction activity of the receptor by the heterologous polypeptide provides a detectable signal mediated by the reporter construct.

49. (Withdrawn) A mixture of recombinant yeast cells, each cell of which has a cell membrane and each cell of which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal;

U.S. Ser. No. 09/581,861
Group Art Unit 1639

a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and

a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of said receptor expressed in the cell membrane;
wherein collectively the mixture of cells expresses a library of said heterologous polypeptides, said library being expressible at a sufficient level such that modulation of the signal transduction activity of said receptor by a heterologous polypeptide within the library provides a detectable signal.

50. (Withdrawn) A mixture of recombinant yeast cells, each cell of which has a cell membrane and each cell of which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal;

a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell;

U.S. Ser. No. 09/581,861
Group Art Unit 1639

a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of said receptor expressed in the cell membrane;

a reporter construct that is activated by the pheromone response pathway; and

a mutation in at least one gene selected from the group consisting of *FAR1*, *SST2*, *BAR1*, *SVG1*, *STE2*, *STE3*, *STE14*, *MFa1*, *MFa2*, *MFa1* and *MFa2*;

wherein the heterologous polypeptide is expressed at a sufficient level such that modulation of the signal transduction activity of the receptor by the heterologous polypeptide provides a detectable signal mediated by the reporter construct.

51. (Cancelled)

52. (Withdrawn) A mixture of recombinant yeast cells, each cell of which has a cell membrane and each cell of which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal;

a chimeric G protein subunit comprising an endogenous yeast G α 1 subunit in which at least the last four C-terminal amino acids of said G α 1 are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, and in which the N-terminus of said G α 1 is operably linked to at least the first five N-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and

U.S. Scr. No. 09/581,861
Group Art Unit 1639

a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of said receptor expressed in the cell membrane;
wherein collectively the mixture of cells expresses a library of said heterologous polypeptides, said library being expressible at a sufficient level such that modulation of the signal transduction activity of said receptor by a heterologous polypeptide within the library provides a detectable signal.

53. (Original) A recombinant yeast cell which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell; and

a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which at least the first five N-terminal amino acids of said Gpa1 are replaced with at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different; such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and
wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

U.S. Ser. No. 09/581,861
Group Art Unit 1639

54. (Original) The yeast cell of claim 53, wherein said chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a first heterologous G protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same.

55. (Withdrawn) The yeast cell of claim 54, wherein said chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a first heterologous G protein subunit, and in which the first 23 N-terminal amino acids of said Gpa1 are replaced with the first 21 N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same.

56. (Cancelled)

57. (Currently Amended) ~~The chimeric G-protein subunit~~ yeast cell of claim [56]1, wherein in said chimeric G protein subunit, the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a heterologous G protein subunit.

58. (Withdrawn) The chimeric G-protein subunit of claim 57, wherein the last six C-terminal amino acids of said Gpa1 are replaced with the last six C-terminal amino acids of a heterologous G protein subunit.

U.S. Ser. No. 09/581,861
Group Art Unit 1639

59. (Original) A chimeric G-protein subunit which comprises an endogenous Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different.

60. (Original) The chimeric G-protein subunit of claim 59, in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of said first heterologous G-protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of said second heterologous G protein subunit.

61. (Withdrawn) The chimeric G-protein subunit of claim 59, in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of said first heterologous G-protein subunit, and in which the first 23 N-terminal amino acids of said Gpa1 are replaced with the first 21 N-terminal amino acids of said second heterologous G protein subunit.

Claims 62-108 (Cancelled).

109. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 107.

U.S. Ser. No. 09/581,861
Group Art Unit 1639

110. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 108.

111. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 109.

112. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 110.

113. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 111.

114. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 112.

115. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 113.

116. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 114.

117. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 115.

U.S. Scr. No. 09/581,861
Group Art Unit 1639

118. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 118.

119. (Withdrawn) The chimeric G protein subunit comprising the amino acid sequence of SEQ ID NO: 123.